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Melilotus officinalis (yellow sweetclover) causes large changes in community and ecosystem processes in both the presence and absence of a cover crop

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Abstract Non-native species are hypothesized to decrease native species establishment and cover crops are hypothesized to decrease non-native species abundance. Although many studies have compared invaded to non-invaded habitats, relatively few studies have experimentally added non-native species to directly examine their effects. In a greenhouse mesocosm experiment, we tested the effects of non-native forbs (*Melilotus officinalis*, *Verbascum thapsus*, and *Lespedeza cuneata*), a proposed C₃ grass cover crop (*Pascopyrum smithii*), and a commonly seeded non-native C₃ grass (*Bromus inermis*) on the establishment of target native C₄ prairie grass species. All treatments contained the same seed density of target C₄ species and were begun on bare soil collected from the field. The legume *M. officinalis* strongly decreased the abundance of all other species, species diversity, and light and soil moisture levels. Surprisingly, *M. officinalis* took up relatively large amounts of labeled

nitrogen (¹⁵N) from the soil early in its development, but *M. officinalis* fixed nitrogen, thus increasing nitrogen in biomass nearly fivefold by the end of the study. We found few effects of either C₃ grass species on non-native forbs or C₄ target species, but seeded *P. smithii* did increase species diversity. Non-native plants therefore impeded native C₄ grass establishment through long-lasting effects of target species seedbank depletion (death of most target seedlings) and altered nutrient availability. The effects of *M. officinalis* were not reduced by the presence of a cover crop.

Keywords Community restoration · Ecological bridge species · Ecosystem functioning · Exotic legume · Invasive species · Nitrogen isotope (¹⁵N)

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Introduction

Assembly rules may constrain species composition such that not all species are able to coexist (Diamond 1975; Fox 1999). For example, many native plants may be unable to coexist with certain non-native species (Christian and Wilson 1999; Brandon et al. 2004; Yurkonis et al. 2005). The relationship between non-native plant abundance and restored plant abundance is generally negative, although not always linear (Brown and Rice 2000; Blumenthal et al. 2003), and high densities of non-native plants can

completely exclude target restoration species (Blumenthal et al. 2003). These studies suggest that native plant restoration may frequently be limited by recruitment rather than by seed availability in sites with high non-native plant abundance. The effects of non-native plants can also persist for long periods (40+ years) after land is abandoned (Christian and Wilson 1999; Dickson et al. 2008). Although non-native species are hypothesized to strongly affect native plants (Wilcove et al. 1998), most of the past studies of the effects of non-native species have been observational (Kareiva 1996). Only recently have researchers begun to experimentally add non-native plants to study their effects on native communities (Aplet and Laven 1993; Brown and Rice 2000; Dukes 2001; Green and Galatowitsch 2002; Corbin and D'Antonio 2004; Raffaele 2004; Adams and Galatowitsch 2008). We directly test the hypothesis that non-native plants cause declines in native plant abundance and diversity by experimentally adding non-native plants to only some treatments.

Non-native species can also affect resource availability, productivity, and species diversity. For example, the non-native legume, *Myrica faya*, increased nitrogen inputs over fivefold on nitrogen-poor volcanic rock, leading to greater growth of the surrounding plants (Vitousek and Walker 1989). Christian and Wilson (1999) and Mack et al. (2000) found that non-native plants can decrease small-scale plant species diversity, but Sax et al. (2002) suggest it may still be possible for non-native plants to increase regional plant richness since the addition of new species often outweighs extinction.

Cover crops are hypothesized to alter plant community assembly by reducing non-native plant abundance and increasing target plant establishment (Fig. 1). Cover crops have been regularly used in agriculture (Hartwig and Ammon 2002), but the

usage of cover crops in restoration is different from their usage in agriculture. In restorations, cover crops must give way to target species and act as a “bridge” to target species dominance (Waldron et al. 2005), whereas in agriculture, they generally do not give way to crop species but are killed with herbicide (Lal et al. 1991). However, the few studies of cover crops in restorations provide mixed results. Cover crops did not decrease *Lythrum salicaria* abundance (Welling and Becker 1993). In other experiments, cover crops provided weed control but did not increase target plant growth (Landhäusser et al. 1996; Pywell et al. 2002; Perry and Galatowitsch 2003). Cover crops were associated with higher species diversity in a comparative study (Martin et al. 2005).

Cover crops are hypothesized to be most effective when they have a similar temporal niche as non-native plants (Fox 1999; Fargione et al. 2003). If cover crops establish before non-native plants, they may exclude non-native plants via a priority effect (Miller 1987). C_3 species actively grow and establish before C_4 species, and in Kansas and many other temperate grassland regions, most non-native plants are C_3 species (USDA and NRCS 2007). Therefore, most proposed cover crops are C_3 species that grow before or concurrently with non-native C_3 plants. One C_3 grass species that is currently being used on government lands as a cover crop is the native species *Pascopyrum smithii* (Palazzo et al. 2003).

Thus far, we have stressed how competition may affect community assembly, but it is possible that facilitation may also play a role. Bare ground can create stressful conditions for plants. Cover crops have been hypothesized to increase target species growth by shading target seedlings and acting as nurse plants (Padilla and Pugnaire 2006) or by preventing soil nutrient loss (Callaway 1995). Facilitation generally occurs in extremely harsh conditions such as deserts and soils with very low nutrients (Bertness and Callaway 1991), but plants can also ameliorate tree seedling stress in somewhat less harsh conditions such as periodically dry grasslands (Ross et al. 2003).

Here, we test the following hypotheses: (1) non-native forbs decrease native target species establishment and (2) the cover crop, *P. smithii*, increases native target species establishment by directly facilitating growth and survival of native target C_4 grasses and by indirectly favoring target species through decreases in non-native forb abundance (reducing the

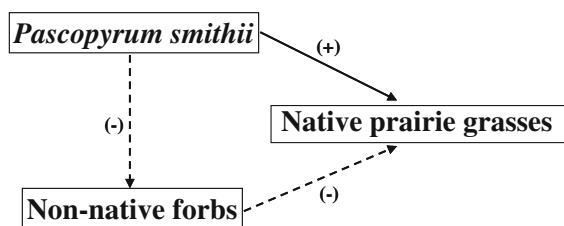


Fig. 1 The predicted relationships between a proposed cover crop (*Pascopyrum smithii*), non-native forbs, and target prairie grasses

negative effects of non-native forbs on native target species; Fig. 1). Our study was performed in a controlled greenhouse experiment, allowing us to add non-native species that could not be added in the field, and allowing us to more tightly focus on plant interactions by removing factors such as herbivory and disturbance.

Materials and methods

Experimental design

We conducted this experiment in a temperature controlled greenhouse (10–37°C) in Ames, Iowa, at Iowa State University. We collected soil in September 2005 from Fort Riley, Kansas (39°30'N, 96°92'W), by removing the top 2 cm of soil in a small upland field and then collecting soil from 2 to 10 cm. The soil was classified as a Wymore–Irwin association of silty clay loam (USDA and KAES 1975). We used field soil to test treatment effects under more realistic conditions. Therefore, seeds, microorganisms, and insects were not removed from the soil. Rhizomes were removed. The soil was placed to a depth of approximately 18 cm in 67 × 44 cm plastic tubs, which is within the range of upland soil depths (5–30 cm) found at nearby Konza Prairie (Benning and Seastedt 1995). Tubs were grouped into three blocks based on whether tubs were in the south, center, or north of the greenhouse. They were watered with tap water to field capacity every 7 days with excess water draining out small holes in the bottom of each tub.

Treatments

We added all species to bare soil on September 30, 2005. We applied a 4 × 3 factorial treatment arrangement in a completely randomized block design, with four C₃ grass treatments (control, seeded *Bromus inermis*, seeded *Pascopyrum smithii*, and *P. smithii* planted as transplants) and three levels of non-native forbs (control, 0.1× density, 1× density). All taxonomy and naming authorities follow USDA and NRCS (2007). Each of the 12 treatments was replicated 3 times for a total of 36 tubs. In a factorial design such as this, there is “hidden replication” (Steel and Torrie 1980). Therefore, main effect tests had nine (C₃ grass) and twelve (non-native density) replicates each.

Barton *P. smithii* cultivar was the native species we tested as a cover crop, and we compared *P. smithii* to a non-native C₃ grass, *B. inermis*, that is often seeded for revegetation after disturbance. We suspect *B. inermis* will have different effects than *P. smithii* because *B. inermis* strongly persists and does not allow target restoration plants to eventually dominate (Dickson et al. 2008).

Pascopyrum smithii was both seeded and planted as transplants to examine priority effects. *Bromus inermis* and *P. smithii* were each seeded at 416 seeds per tub (0.93 and 1.74 g, respectively). Transplants had not tillered and were each 0.01 g dry weight and 26 days old when added to tubs. Twenty transplants were evenly spaced in appropriate tubs.

Non-native forb treatments were applied by seeding a mix of three different species, the annual/biennial *Melilotus officinalis*, the biennial *Verbascum thapsus*, and the perennial *Lespedeza cuneata*. The mix was seeded at 140 seeds of each species per tub at 1× density (0.30, 0.01, and 0.19 g, respectively) and at 14 seeds of each species per tub at 0.1× density. These non-native forb species were used because they were commonly found at Fort Riley and land managers perceived them to be important. Non-native forb seeding densities were selected to bracket the observed range of natural densities in the field (Gross 1980; Klemow and Raynal 1981).

Target species

Each tub received the same C₄ prairie grass (target species) seed mix. We added target C₄ species at different amounts to mimic the practice of seeding species such as *S. nutans* and *A. gerardii* at lower densities than species such as *S. scoparium* and *B. curtipendula* (Weber 1999). Every tub received the following amounts of C₄ grass cultivar seed: 25 seeds (0.07 g) of Cheyenne *Sorghastrum nutans*; 43 seeds (0.12 g) of Kaw *Andropogon gerardii*; 112 seeds (0.20 g) of Aldous *Schizachyrium scoparium*; and 237 seeds (0.21 g) of El Reno *Bouteloua curtipendula*.

Sampling design

We collected data on variables related to initial establishment, adult percent coverage, net primary productivity, resource levels, and species diversity measures. After seedlings emerged, we counted the

number of seedlings of each sown species in each tub. We sampled the percent coverage of bare ground, litter, and each live species in every tub in January, March, and June 2006. At the end of June, after most C₄ grass individuals had flowered and set seed, we clipped all aboveground biomass to ground level and sorted all identifiable live and dead biomass to species. We therefore measured productivity because we collected all biomass (live and dead) that had been produced during the study. Root biomass was collected by taking two 4.7 cm diameter cylindrical soil cores to the full depth of soil in the tub. Root-free soil was collected from these cores at all soil depths for ¹⁵N analysis (see below). Soil was then washed from the roots using tap water and three successively smaller sieves (4.0, 2.0, and 0.3 mm openings). Aboveground biomass and root biomass was dried at 75°C for 96 h before being weighed.

Light levels were collected between 11 am and 1 pm CST in December 2005, and in January, March, and May 2006 using a PAR-80 ceptometer from Decagon Devices, Inc. (Pullman, WA, USA) with an external Li-Cor Quantum sensor (Lincoln, NE, USA) to simultaneously measure above- and below-canopy light levels. Soil moisture was measured gravimetrically (weight of water in soil/weight of wet soil) at the soil surface by collecting 3 cm deep soil samples in December 2005 and March and June 2006.

In experimental designs where the presence and absence of non-native and cover crop species are varied, the presence or absence of these species will affect the initial species richness and diversity values, and this must be taken into account in analyses. To account for these initial differences in the richness and diversity of the plantings, we examined the changes in species richness and diversity from the time of initial planting to the end of the experiment, rather than just examining richness and diversity at the end of the experiment. We calculated the change in species richness or diversity as the log response ratio (Goldberg et al. 1999) of the richness or diversity of final live aboveground biomass divided by the richness or diversity of the initial seed and transplant biomass (initial seed and transplant weights are listed above).

¹⁵N study

We conducted a ¹⁵N tracer study to better understand the uptake and loss of soil nitrogen. After a canopy

had developed (December 14, 2005), we injected 0.547 g of 98+% pure ¹⁵NH₄¹⁵NO₃ dissolved in 280 ml of distilled water into the top layer of soil in each tub. We ground the June 2006 biomass to a powder and combined live and dead biomass from each tub into the following groups for ¹⁵N analysis: *M. officinalis*, all sown non-native species except *M. officinalis*, roots, soil containing no roots, all dead vegetation that could not be identified to species (litter), all species that came from the seedbank, *B. curtipendula*, all other sown C₄ prairie species, *B. inermis*, and *P. smithii*. Samples were analyzed for % N and % ¹⁵N at the North Carolina State University Analytical Services Lab using a Thermo Finnigan DELTA plus mass spectrometer (nitrogen uptake for each group was estimated as: ¹⁵N in a particular group/total added ¹⁵N). The percentage of ¹⁵N lost (mostly leached) from each tub was calculated by subtracting the ¹⁵N in all the groups from the total amount of added ¹⁵N.

Statistical analysis

All data analyses were completed in SAS for Windows 8.02 (see Appendix S1 in the supplementary material). Type III sum of squares were used in all analyses, and we used a *P* = 0.05 level of significance (**P* < 0.05, ***P* < 0.01, ****P* < 0.001). All unmentioned treatment effects and contrasts were not significant. The blocking term was included to remove spatial variation, although blocking term results are not reported.

Our specific predictions (and how we tested them with a priori contrasts) are as follows: (1) increasingly higher sowing densities of non-native forbs will increasingly reduce native C₄ grass abundance (linear and quadratic contrasts between non-native forb sowing density [0×, 0.1×, 1×] and C₄ grass abundance); (2) the presence of C₃ grasses will reduce non-native forb abundance (contrast between treatments with and without seeded C₃ grasses); (3) *Pascopyrum smithii* transplants will decrease non-native forb abundance more than seeded *P. smithii* due to a priority effect (contrast between seeded *P. smithii* and *P. smithii* transplant treatments); (4) *Pascopyrum smithii* and *B. inermis* will have different effects on C₄ grass abundance (contrast between seeded *B. inermis* and seeded *P. smithii* treatments); and (5) *Pascopyrum smithii* will directly facilitate the establishment of target C₄ grasses, whereas

B. inermis will compete too much with C_4 grasses to show facilitation (we analyze the effects of *P. smithii* and *B. inermis* in treatments without *M. officinalis* to examine the direct, possibly facilitative, effects of C_3 grasses).

Non-native forb and C_3 grass percent coverage and biomass was equal to zero in the non-native forb and C_3 grass control treatments, respectively, and thus could not be transformed to normality. To allow parametric analyses, we removed the treatment with no sown non-native forbs when analyzing non-native forb percent coverage and biomass, and we removed the treatment with no C_3 grasses when analyzing C_3 grass percent coverage and biomass. We report medians in the text for data that needed to be transformed, and we report means in the text for data that were normally distributed and untransformed (see Appendix S2 in the supplementary material).

Results

Seedling emergence

There was high emergence of all sown species 14 days after seeds were sown, suggesting that subsequent treatment effects were not due to a lack of seedling emergence (see Appendix S3 in the supplementary material).

Non-native forb productivity and percent coverage

Melilotus officinalis made up 97 and 100% of the total sown non-native forb productivity (1.74 and $2.08 \text{ g m}^{-2} \text{ day}^{-1}$) at $0.1\times$ and $1\times$ non-native forb density, respectively. We hereafter refer to the sown non-native forb treatments as the *M. officinalis* treatments because the two are essentially synonymous. Across time periods, higher *M. officinalis* sowing densities increased *M. officinalis* percent coverage more in January and June than in March (time \times *M. officinalis* interaction; Tables 1, 2).

Even though C_3 grass treatments had no significant effect on *M. officinalis* aboveground productivity, they did cause declines in percent coverage in some months. C_3 grass treatments caused slightly lower *M. officinalis* percent coverage in January and June, but had no significant effect in March (time \times C_3 grass interaction; Tables 1, 2).

C_3 grass productivity and percent coverage

Higher *M. officinalis* sowing densities significantly decreased C_3 grass aboveground productivity ($F_{2, 16} = 5.0^*$) in a linear manner (contrast: $F_{1, 16} = 6.4^*$) such that mean productivity was 0.28 , 0.35 , and $0.22 \text{ g m}^{-2} \text{ day}^{-1}$ at $0\times$, $0.1\times$, and $1\times$ *M. officinalis* sowing density, respectively. Higher sowing densities of *M. officinalis* decreased C_3 grass percent coverage in January and March, but actually led to a slight increase in C_3 grass percent coverage in June (time \times *M. officinalis* interaction; Tables 1, 2).

Even though there were no significant differences between *B. inermis* and *P. smithii* aboveground productivity, there were differences in percent coverage during some months. *Bromus inermis* percent coverage was higher than *P. smithii* percent coverage during the early parts of the experiment, but their coverage tended to converge over time (time \times C_3 grass interaction; Tables 1, 2).

Target prairie grass percent coverage and productivity

Higher sowing densities of *M. officinalis* dramatically reduced the increase in sown prairie grass percent coverage over time (time \times *M. officinalis* interaction; Table 1; Fig. 2a), and dramatically reduced total sown C_4 grass aboveground productivity (Fig. 2b). Median aboveground productivity of *B. curtipendula* was 0.12 , 0.15 , and $0.01 \text{ g m}^{-2} \text{ day}^{-1}$ at $0\times$, $0.1\times$, and $1\times$ *M. officinalis* sowing density, respectively. The median aboveground productivity of non-*B. curtipendula* sown grasses was even more dramatically reduced, with 0.19 , 0.03 , and $0 \text{ g m}^{-2} \text{ day}^{-1}$ at $0\times$, $0.1\times$, and $1\times$ *M. officinalis* sowing density, respectively.

Even though C_3 grass treatments had no significant effect on sown C_4 grass aboveground productivity, C_3 grasses in June unexpectedly decreased prairie grass percent coverage from its already low coverage at $1\times$ *M. officinalis* density (time \times C_3 grass \times *M. officinalis* interaction; Table 1; Fig. 2a).

We also analyzed the effects of C_3 grasses in treatments without *M. officinalis*. In the absence of *M. officinalis*, C_3 grasses had no significant effect on sown C_4 grass percent coverage or aboveground productivity, suggesting C_3 grasses did not facilitate C_4 grass establishment (Fig. 1).

Table 1 Results of repeated measures analyses

Treatment	Sown non-native forb % coverage	Sown C ₃ grass % coverage	Sown C ₄ grass % coverage	Soil moisture	Light penetration
<i>Between-subjects</i>					
C ₃ Grass (C ₃)	$F_{3, 14} = 1.9$	$F_{2, 16} = 25.6^{***}$	$F_{3, 22} = 0.8$	$F_{3, 22} = 0.1$	$F_{3, 22} = 1.6$
No C ₃ vs. seeded C ₃	$F_{1, 14} = 2.7$	NA	$F_{1, 22} = 0.2$	$F_{1, 22} = 0.1$	$F_{1, 22} = 1.5$
<i>B. inermis</i> seeded vs. <i>P. smithii</i> seeded	$F_{1, 14} = 2.5$	$F_{1, 16} = 32.7^{***}$	$F_{1, 22} = 0.3$	$F_{1, 22} = 0.2$	$F_{1, 22} = 2.9$
<i>P. smithii</i> seeded vs. <i>P. smithii</i> transplants	$F_{1, 14} = 0.7$	$F_{1, 16} = 0.7$	$F_{1, 22} = 0.9$	$F_{1, 22} = 0.0$	$F_{1, 22} = 0.0$
<i>M. officinalis</i> (<i>M.o.</i>)	$F_{1, 14} = 12.2^{**}$	$F_{2, 16} = 11.6^{***}$	$F_{2, 22} = 22.6^{***}$	$F_{2, 22} = 5.7^*$	$F_{2, 22} = 73.8^{***}$
Linear density effect	NA	$F_{1, 16} = 23.0^{***}$	$F_{1, 22} = 40.7^{***}$	$F_{1, 22} = 10.7^{**}$	$F_{1, 22} = 124.2^{***}$
Quadratic density effect	NA	$F_{1, 16} = 0.2$	$F_{1, 22} = 4.6^*$	$F_{1, 22} = 0.8$	$F_{1, 22} = 23.4^{***}$
C ₃ × <i>M.o.</i>	$F_{3, 14} = 1.9$	$F_{4, 16} = 1.5$	$F_{6, 22} = 1.3$	$F_{6, 22} = 0.3$	$F_{6, 22} = 2.9^*$
<i>Within-subjects</i>					
Time (<i>T</i>)	$F_{2, 28} = 125.4^{***}$	$F_{2, 32} = 18.3^{***}$	$F_{2, 44} = 107.8^{***}$	$F_{2, 21} = 454.5^{***}$	$F_{3, 66} = 9.7^{***}$
<i>T</i> × C ₃	$F_{6, 28} = 2.9^*$	$F_{4, 32} = 5.9^{**}$	$F_{6, 44} = 1.1$	$F_{6, 26.2} = 0.8$	$F_{9, 66} = 0.4$
<i>T</i> × <i>M.o.</i>	$F_{2, 28} = 7.5^{**}$	$F_{4, 32} = 12.9^{***}$	$F_{4, 44} = 10.2^{***}$	$F_{4, 24.4} = 10.4^{***}$	$F_{6, 66} = 9.2^{***}$
<i>T</i> × C ₃ × <i>M.o.</i>	$F_{6, 28} = 1.3$	$F_{8, 32} = 1.5$	$F_{12, 44} = 2.9^{**}$	$F_{12, 27.7} = 1.5$	$F_{18, 66} = 0.9$

NA refers to tests that are not applicable because the treatments without sown non-native forbs and sown C₃ grass cannot be included in the specified analyses

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2 Percent coverage of *Melilotus officinalis* and sown C₃ grasses, as measured in 3 months in 2006

	<i>M. officinalis</i>			C ₃ grasses			Light penetration
	January	March	June	January	March	June	Average
No C ₃ ; 0×	0	0	0	0	0	0	54
B. seed; 0×	0	0	0	51	30	9	21
P. seed; 0×	0	0	0	17	22	3	35
P. trans.; 0×	0	0	0	9	11	2	24
No C ₃ ; 0.1×	88	50	86	0	0	0	11
B. seed; 0.1×	76	36	82	39	22	21	10
P. seed; 0.1×	58	37	69	14	11	3	11
P. trans.; 0.1×	55	46	64	8	11	11	19
No C ₃ ; 1×	92	40	80	0	0	0	5
B. seed; 1×	82	45	85	19	1	17	4
P. seed; 1×	88	35	83	8	1	6	3
P. trans.; 1×	92	52	80	3	4	9	6

Also shown is the percent light penetration averaged across four sampling periods. Values are medians

Net primary productivity

Higher sowing densities of *M. officinalis* significantly increased total aboveground productivity ($F_{2, 22} = 40.1^{***}$), with *M. officinalis* making up 69 and 77% of total aboveground productivity at 0.1× and 1× *M. officinalis* density, respectively. A large increase

in mean productivity occurred between 0× and 0.1× *M. officinalis* density (1.63 and 2.54 g m⁻² day⁻¹, respectively) and a much smaller increase occurred when *M. officinalis* density increased to 1× (2.71 g m⁻² day⁻¹; linear contrast: $F_{1, 22} = 38.7^{***}$; quadratic contrast: $F_{1, 22} = 41.5^{***}$). In contrast, *Melilotus officinalis* sowing density did not significantly affect

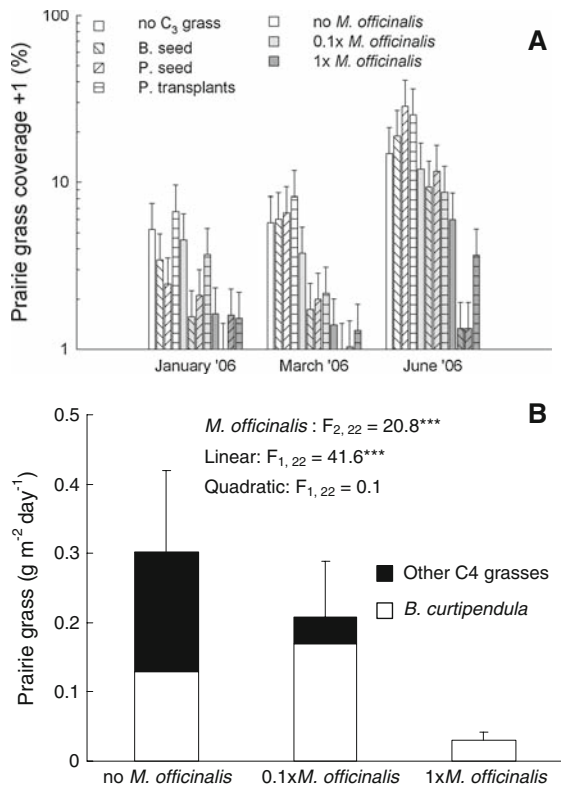


Fig. 2 The percent coverage (+1) of sown C₄ prairie grasses (a) and the aboveground productivity of sown C₄ prairie grasses produced during the study (b). The error bars are +1 standard error and the scale in (a) is logarithmic (B. is *B. inermis* and P. is *P. smithii*; ****P* < 0.001)

root productivity ($1.21 \text{ g m}^{-2} \text{ day}^{-1}$ median). The C₃ grass treatments did not significantly affect total root or aboveground productivity.

¹⁵N uptake and total N

No treatment significantly affected the percentage of ¹⁵N (labeled nitrogen) that leached from the tubs (33.8% mean), stayed in the soil (33.3% mean), or was found in the roots (11.2% median). *Melilotus officinalis* density significantly affected the amount of ¹⁵N in aboveground vegetation (Fig. 3a) with sown *M. officinalis* taking up much of the ¹⁵N at $1 \times$ *M. officinalis* sowing density and species from the seedbank taking up much of the ¹⁵N in the $0 \times$ *M. officinalis* sowing treatment. C₃ grasses did not significantly affect the amount of ¹⁵N in aboveground vegetation.

Higher sowing densities of *M. officinalis* significantly increased total nitrogen in combined aboveground and

root biomass ($F_{2,22} = 55.1^{***}$) such that a very large increase in median nitrogen occurred between $0 \times$ and $0.1 \times$ *M. officinalis* density (4.5 and 24.9 g m^{-2} , respectively) but no increase occurred when *M. officinalis* density increased to $1 \times$ (23.0 g m^{-2} ; linear contrast: $F_{1,22} = 40.9^{***}$; quadratic contrast: $F_{1,22} = 69.4^{***}$). C₃ grasses had no significant effects on total nitrogen in combined aboveground and root biomass.

Water and light availability

Higher *M. officinalis* sowing densities decreased soil moisture in December and June, but had little effect on soil moisture in March (time \times *M. officinalis* interaction; Table 1; Fig. 3b). Higher *M. officinalis* sowing densities also decreased light penetration in general, but had little effect on light penetration in March, likely because the canopy of *M. officinalis* briefly died back (time \times *M. officinalis* interaction; Table 1; Fig. 3c). Averaged across time periods, C₃ grasses generally decreased light penetration in treatments without sown *M. officinalis*, but increased or had little effect on light penetration when *M. officinalis* was sown (C₃ grass \times *M. officinalis* interaction; Tables 1, 2).

Species diversity

Melilotus officinalis caused large saturating declines in species richness and diversity, but richness and diversity tended to increase over time in the absence of *M. officinalis*. Richness and diversity declined more from $0 \times$ to $0.1 \times$ *M. officinalis* sowing density than from $0.1 \times$ to $1 \times$ density (richness linear contrast: $F_{1,22} = 209.8^{***}$, quadratic contrast: $F_{1,22} = 74.4^{***}$, Fig. 4a; diversity linear contrast: $F_{1,22} = 85.7^{***}$, quadratic contrast: $F_{1,22} = 19.5^{***}$, Fig. 4b).

Treatments with no C₃ grasses showed a decline in species richness (contrast: $F_{1,22} = 5.8^*$; Fig. 4a) and diversity (contrast: $F_{1,22} = 36.5^{***}$; Fig. 4b) relative to treatments where C₃ grasses were seeded. Treatments with seeded *B. inermis* showed a decline in richness (contrast: $F_{1,22} = 8.9^{**}$) and diversity (contrast: $F_{1,22} = 5.7^*$) relative to treatments with seeded *P. smithii*, even though the effect of *B. inermis* on richness changed at different *M. officinalis* densities. The seeded *B. inermis* treatment showed a slight increase in richness relative to other C₃ grass

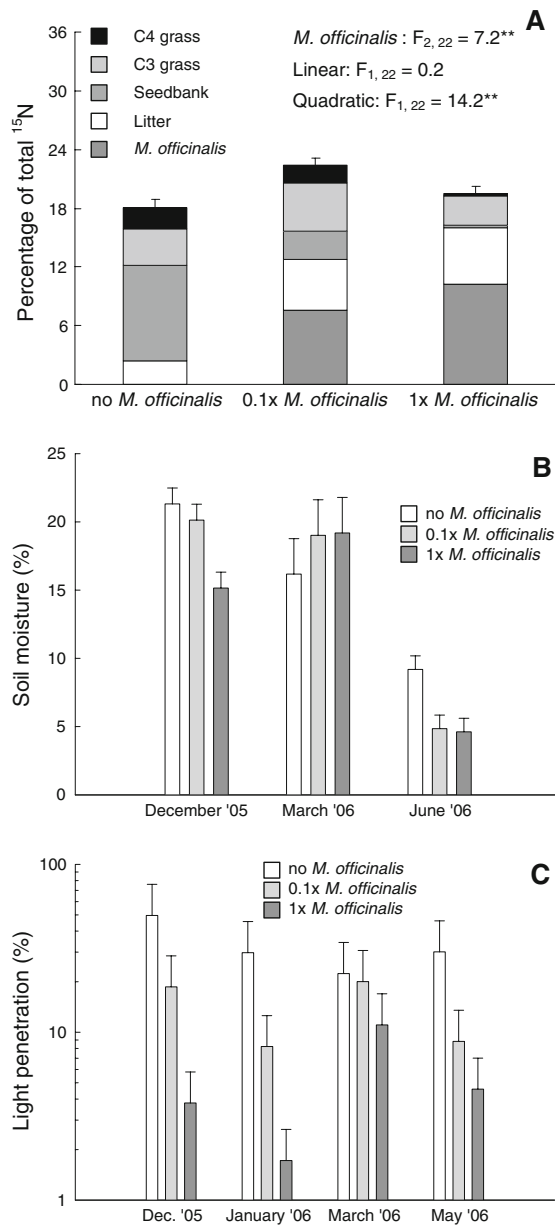


Fig. 3 The percentage of total added ^{15}N taken up by different types of aboveground biomass (a), percentage soil moisture by weight (b), and percentage light penetration (c). The error bars are ± 1 standard error and the scale in (c) is logarithmic ($^{**}P < 0.01$)

treatments at 0x *M. officinalis* density but a decrease at 0.1x and 1x density (*C*₃ grass x *M. officinalis* interaction). Treatments with transplanted *P. smithii* showed a decline in diversity (contrast: $F_{1,22} = 36.2^{***}$) relative to treatments with seeded *P. smithii*, largely because the initial weight of *P. smithii*

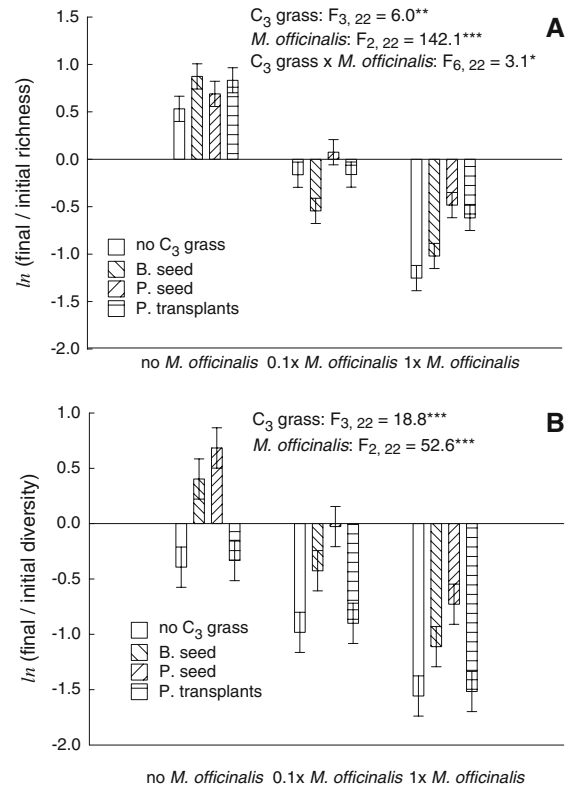


Fig. 4 Change in species richness (a) and diversity (b) as a function of *Melilotus* treatments. Error bars are ± 1 standard error (B. is *B. inermis* and P. is *P. smithii*; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

transplants (0.20 g) was more similar to the added weight of other species than *P. smithii* seeds (1.74 g), thereby causing the transplant treatment initial diversity to be higher than the seed treatment initial diversity.

Discussion

The non-native forb *Melilotus officinalis* strongly decreased the abundance of all other species in this controlled greenhouse experiment. Our results suggest that non-native legumes can be the main driver of community and ecosystem processes under certain conditions. In comparison, the cover crop, *Pascopyrum smithii*, and *Bromus inermis* had little effect on *M. officinalis* abundance, target prairie grass abundance, or any other response variable except species richness and diversity. We therefore reject

our general cover crop hypothesis (Fig. 1), leaving only the direct effect of non-native forbs.

Melilotus officinalis grew quickly after germination, overtopped other species, and limited the amount of light available to other species. Several months after germination, this non-native legume also began to limit soil moisture availability by taking up large amounts of water to supply its large canopy, and by March 2006, it briefly thinned its canopy. *Melilotus officinalis* took up fairly large amounts of ^{15}N , suggesting it competed for soil nitrogen. This was somewhat surprising, since legumes harbor N-fixing bacteria. *Melilotus officinalis* likely competed most strongly for nitrogen in the early stages of its development when legumes primarily take up unfixed soil nitrogen (Vallis et al. 1977) and before root nodules have developed (Wolf and Rohrs 2001). Therefore, this non-native legume competed for all the resources that we measured, even nitrogen. Nevertheless, *M. officinalis* ultimately increased the amount of nitrogen in the system through nitrogen fixation. *Melilotus officinalis* increased plant production over 1.5-fold and increased the total amount of nitrogen in belowground and aboveground biomass over 4.5-fold when comparing treatments with no sown *M. officinalis* to treatments with $1\times$ sowing density. Once this biomass begins decomposing, it should increase nitrogen availability for some time (Ranells and Wagger 1996). Higher nitrogen availability could affect species composition in the long run, and this should be tested in longer term legume invasion studies.

Strong effects of *M. officinalis* on species composition and nutrient supply may be common. *Melilotus officinalis* has a nearly worldwide distribution (USDA et al. 2008) and is present in all 50 states in the USA (USDA and NRCS 2007). Field studies have found *Melilotus* species at densities comparable to this study (Turkington et al. 1978; Klemow and Raynal 1981), and *M. officinalis* is found in near monoculture at some heavily disturbed locations at Fort Riley, Kansas, but is generally at much lower abundance in undisturbed locations (personal observations). Still, few studies have examined the effects of *M. officinalis*, and this is the first experimental study of *M. officinalis*. *Melilotus officinalis* is an annual or biennial plant (Turkington et al. 1978), and does not generally dominate perennial grasslands over long periods of time (Rebele 1992), but its

effects on community and ecosystem processes may persist for long time periods.

It is often assumed that sown plants will eventually establish reproducing populations in restorations (Schramm 1992), but this will not happen if their seedlings die after emerging. In our study, prairie grasses germinated in the presence of non-native forbs and then nearly all prairie grass individuals died over time. Non-native species may therefore deplete the seedbank, which could have long lasting effects on plant community dynamics. A loss of the original seedbank has been shown to lead to a different plant community trajectory (Pakeman and Small 2005).

The large effects of *M. officinalis* suggest non-native plants must be controlled for restorations to be successful. We predicted the cover crop, *P. smithii*, would grow quickly and promote C_4 prairie grass establishment directly via facilitation and indirectly via non-native forb control (Fig. 1). *Pascopyrum smithii* did establish quickly but it had little effect on *M. officinalis* growth, and it did not appear to facilitate C_4 prairie grass establishment in the absence of *M. officinalis*. Furthermore, there was evidence that sown C_3 grasses competed with C_4 prairie grasses in the presence of *M. officinalis*, as suggested by a time \times C_3 grass \times *M. officinalis* interaction on C_4 grass percent coverage. There was little evidence that *P. smithii* affected target C_4 grasses differently than *B. inermis*. There was also little evidence of a priority effect of *P. smithii* since both seeded and transplanted *P. smithii* had similarly small effects on *M. officinalis* or C_4 prairie grasses. The lack of a priority effect may be due to *P. smithii* transplants showing less tillering and lateral spread than we expected. It therefore appears that *B. inermis* and *P. smithii* are not good candidates to control non-native forb abundance or increase C_4 prairie grass establishment under the conditions of this study. Nevertheless, it is possible that subhumid grassland conditions are not harsh enough for any facilitative effects of *P. smithii* to outweigh its competitive effects (Choler et al. 2001; Padilla and Pugnaire 2006).

Seeded C_3 grasses did increase community species richness and diversity in our study. Martin et al. (2005) also found a positive association between the C_3 cover crop (*Elymus canadensis*) and species richness and diversity, but their cover crop was seeded in different locations of the site. Therefore,

the cover crop effect was confounded with geographical location. The cover crop effect in our study was not confounded with any other variable, suggesting that cover crops may have the potential to increase species richness and diversity. Still, it should be remembered that even though seeded C_3 grasses increased species richness and diversity, *M. officinalis* caused a proportionally larger decrease in richness and diversity. The effects of *P. smithii* and *B. inermis* on species richness and diversity may have been due to a slight decrease in the early establishment of *M. officinalis*, as suggested by a time \times C_3 grass interaction for *M. officinalis* percent coverage. However, this is only a tentative hypothesis.

The conditions of this study were obviously different from field conditions, even though we used field soil, did not fertilize, and allowed water limitation to occur. Still, several general conclusions can be taken from this study. First, under certain conditions, an invasive non-native species such as *M. officinalis* may be able to outcompete native C_4 prairie grasses. Thus, a purely competition-colonization tradeoff model does not adequately describe our results (Pacala and Rees 1998) because late successional grasses were present but were not able to establish at the start of succession in the presence of *M. officinalis*. It should also be noted that *B. curtipendula*, which typically established more quickly than the other C_4 grasses in this study, grew much better than the other C_4 grasses in the presence of *M. officinalis*, suggesting that individuals may need to grow quickly to compete with *M. officinalis*. Second, our study differs from most theoretical models because many models assume that locally extinct species will be able to colonize in the future from the seedbank or from dispersal. However, non-native species may deplete the native seedbank by excluding native C_4 grasses after they germinate, thus causing establishment to fail after one-time seed dispersal events. Even fairly low densities of non-native species can strongly affect recruitment, since the effects of $0.1 \times$ sowing density were often similar to the effects of $1 \times$ sowing density. Therefore, aggressive non-native species must be controlled at the start of restoration or succession if native dominance is the goal. Lastly, cover crops such as *P. smithii* may not be able to substantially control non-native forbs such as *M. officinalis*, and *P. smithii* may not substantially increase target species

abundance. Still, seeded *P. smithii* may have some value as a cover crop by increasing the diversity of species from the seedbank, even though further work is necessary to determine the mechanism whereby *P. smithii* may increase species diversity.

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